Volume 15 Number 6 1987 Nucleic Acids Research

Identification of an Epstein–Barr virus-specific desoxyribonuclease gene using complementary DNA

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Received November 20, 1986; Revised and Accepted February 17, 1987

ABSTRACT

We have recently obtained 18 distinct cDNA clones representing different genes expressed in the early phase of EBV infection. One of them, c37, which is situated at the position 12907-122451 in the B95-8 viral genome, is shown here to code for a viral desoxyribonuclease [DNase]. Cell free translation of c37-selected messenger RNA yielded a protein of about 52 KDa which was immunoprecipitated by a high EA titer serum from nasopharyngeal carcinoma patient. This protein showed a DNase activity which was resistant to high salt concentrations (150 to 300mM KCl) and was specifically neutralized by EA positive serum. These properties are typical of the EBV-specific DNase activity that we recently described in chemically induced EBV-transformed lymphoid cells. The same results were obtained on cell-free translation of the native RNA synthesized in vitro from pGEM-37 plasmid containing the entire c37 cDNA sequence (1.53 Kb). These data indicate that the BGLF5 open reading frame contained in c37 encodes for the EBV-specific DNase.

INTRODUCTION

Epstein-Barr virus (EBV), an ubiquitous human herpesvirus, causative agent of infectious mononucleosis has been implicated in two human cancers : Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC). Additionally, EBV is capable of immortalizing primate or human B lymphocytes into continuously proliferating cell lines carrying the EBV genome (1,2,3).

Although the expression of the viral genome is repressed and limited to a latent state in most infected cells, a productive viral cycle can be induced in some cell lines. In a substantial fraction of latently infected cells, treatment by 12-O-tetradecanoylphorbol-13-acetate (TPA) (4) and by sodium butyrate (SB) (5) leads to virus replication, as characterized by the successive appearance of early antigen (EA) in the early stage, viral capsid antigen (VCA) at the late stage, and finally, production of viral particles.

A viral DNase activity has been detected during the early stage both in reactivated and EBV-positive lymphoid cell lines (6,7,8). The viral enzyme activity may be neutralized by early antigen positive sera from NPC patients (7,8,9) and the degree of anti-DNase activity correlates with EA antibody titers (8), indicating that viral DNase is one of the constituants of the EA complex. Significantly elevated,

anti-DNase antibody titers in NPC patients might imply reactivation of the viral genome and so their titre could be valuable as a prognostic element. Viral DNase appears to be essential for DNA synthesis, repair, recombination and virus replication (9,10). Biochemical studies of a DNase from HSV showed that this enzyme has endonuclease activity and both 5' and 3' exonuclease activities (11,12) though its precise role in viral infection is not yet clear. We therefore attempted the identification and characterization of the EBV-specific DNase gene as a first step for determining its role in EBV infection and replication in vivo. The entire EBV genome has recently been sequenced (13), however, the DNase gene has not been precisely located, although a weak amino acid sequence homology between the HSV exonuclease gene and an EBV Bam HI B/G fragment has recently been described by Mc Geoch et al. (14). We have attempted to localize and characterize some early EBV genes using a library of complementary DNA to mRNAs from P3HR-1 cells expressing only early viral functions (15).

The present report concerns one cDNA clone which allowed us to localize a typical viral DNase on the Bam HI B and G fragments of EBV genome. This finding should facilitate further characterization of the enzyme and the understanding of its role in EBV infection.

MATERIALS AND METHODS

(a) Lymphoid cell culture and EBV induction with TPA plus SB

The EBV-producer P3HR-1 line was propagated by seeding $5x10^5$ cells per ml in RPMI 1640 medium supplemented with 10 % heat-inactived foetal calf serum, 100 U of penicillin per ml, and 250µg of streptomycin per ml, and grown at 37° C. The viral cycle in P3HR-1 cells was induced by adding 20 ng of TPA per ml and 2mM SB (8). Simultaneously cells were treated with 50µg of araT (arabinofuranosylthymine) or 200mM PAA (phosphonoacetic acid) to limit the viral cycle to the early stage only (17). Cells were harvested 3 days later and total cytoplasmic RNAs were extracted. The percentage of EA-positive cells was evaluated on smears using the indirect immunofluorescence test (18).

(b) RNA extraction and RNA blot

Cytoplasmic RNA was prepared by the Guanidinium/cesium chloride method (19). Briefly, 10^9 P3HR-1 cells were collected and washed with cold phosphate buffered saline (PBS), resuspended in low salt buffer (10mM Tris-HCl, pH : 7.4, 10mM NaCl, 3mM MgAc and 10mM vanadyl-ribonucleoside complex : VRC) and then the cells were lysed by the addition of 1/3 volume of low salt buffer containing 5 % sucrose (w/v), and 2 % Nonidet 40 (v/v). The suspended cells were homogenized 10 times with a Dounce homogenizer. The nuclei were removed by centrifugation at 1000g for

5 min. The cytoplasmic extract was mixed with 5 vol. of a solution containing 0.7M -mercaptoethanol, 2 % sarkosyl and 6M guanidine thiocyanate and was layered on a cushion of 5.7M CsCl in a centrifuge tube. After centrifugation at 100000g for 18 hours, the RNA pellet was resuspended in 7M guanidine hydrochloride and precipitated by adding 1M acetic acid and ethanol.

mRNA was selected by passing total cytoplasmic RNA for 4 times through an oligo-dT-cellulose column.

Northern blot hybridization was done as described previously (15), except that genescreen sheets (NEN) were used instead of nitrocellulose.

(c) Hybrid-selection of RNA

250 micrograms of each cDNA clone from the P3HR-1 strain were digested with EcoRI restriction enzyme and immobilized on 1 cm^2 pieces of diazobenzyloxymethyl-paper (20). Complementary RNA was selected by hybridization in a solution containing 50 % formamide, 0.1 % SDS, 0.6 mM NaCl, 4 mM EDTA, 80 mM Tris-HCl (pH 7.8)and 1.5 mg of polyadenylated RNA per ml (19).

(d) Cell-free translation, immunoprecipitation, gel electrophoresis and fluorography

Hybrid-selected RNAs were translated in the message-dependent rabbit reticulocyte system (Amersham, U.K.). An aliquot of 117µl, containing 10µl of RNA, 100µl of rabbit reticulocyte lysate and 100µCi of (^{35}S)-Methionine, was incubated for 90 min. at 30° C. After incubation, 2µl of 100mM PMSF (phenylmethylsulfonyl Fluoride) and 10µl of EA positive serum TU115 (VCA titer : 2580 ; EA titer : 2580 ; EBNA titer : 160) were added and incubated for one night at 4° C. 200 µl of Staphylococcus suspension(Institut Mérieux, Lyon) were then added to this mixture and incubated for 30 min. at 4° C. After centrifugation, the pellets were washed 3 times with NET (NaCl 150mM, 5mM EDTA, 50mM tris-HCl, pH : 7.4 and 0.2 % Azide) plus 0.5 % Nonidet 40 and finally 3 times with NET. Immunoprecipitated protein fractions were analyzed on $NaDodSO_4$/polyacrylamide gels as described by Laemmli (21)

(e) DNase activity assays

EBV-specific DNase activity was measured as described previously (8). Briefly a 83µl final assay volume consisted of 27.5µl of the tranlation mixture described above, a final concentration of 3mM $MgCl_2$, 10ng DNA corresponding to 27000 cpm ($2.7x10^6$ cpm/µg DNA nick-translated with ^{32}P-dCTP), 1mM dithiothreitol and 50mM TRIS-hydrochloride (pH : 8.0). The reactions were performed in the presence of 150mM KCl, at which concentration cellular DNase was approximately 90 % inhibited while EBV specific DNase activity was unaffected. After incubation for 2 hours at 37° C the reaction was stopped by adding 50µg of salmon testis DNA and 20mM EDTA, and

the products tranfered onto Whatman GF/C glass fiber disks. The acid-insoluble fraction was precipitated with a 10 % cold Trichloroacetic acid solution. The disks were dried with ethanol and ether, and counted in a Packard scintillation counter. Specific inhibition of EBV DNase activity by a high EA titer serum was tested by incubating the translated solution with 10µl of anti-EA serum or control serum for 30 min. at room temperature before the enzymatic reaction.

(f) Synthesis of native RNA from cDNA sequence

Large amounts of capped RNA have been produced from the cDNAs using the Riboprobe gemini TM system (Promega Biotec, Wisconsin, USA). The c37 plasmid was treated with Pst-I restriction enzyme in order to obtain the cDNA sequence which was then inserted into pGEMI plasmid (called pGEM-37) containing SP 6 and T7 RNA polymerase promotors. Linearized pGEM-37 (5µg) was incubated in a solution containing 40mM Tris-HCl (pH : 7.5) 6mM MgCl2, 1mM Spermidine, 5mM NaCl, 10mM DDT, 100µg/ml of BSA, 0.5mM of each ATP, CTP, UTP, 0.05mM GTP, 0.5mM GpppG and 50 units of SP 6 or T7 RNA polymerases at 37° C for 1-1.5 hours. The DNA template was removed from the transcription mixture by treatment with RQI DNase (Promega Biotec) at 37° C for 15 min. Capped RNA was extracted twice with phenol- chloroform (1:1), washed 3 times with ether and precipitated with 0.3M Na-acetate (pH : 5.2) and ethanol.

The quantity and purity of the synthesized RNA were determined by electrophoresis on RNA gel (15) and checked by UV transillumination. With 10µg of pGEM-37 we obtained about 6µg of pure 1.5 kb RNA from both promotors.

(g) DNA sequence analysis

The sequences of both extremities of c37 were analyzed using both GEMSEQ dsDNA sequencing (Promega, Biotec) and the method of Maxam and Gilbert (22). For the GEMSEQ sequencing system, pGEM-37 was linearized by digestion with EcoRI or Hind III enzymes and then annealed respectively with SP 6 or T7 primer at 100° C for 3 min. in 10mM Tris-HCl (pH : 7.5) and 5mM NaCl. ^{32}P-dATP was added to the above mixture and then pGEM-37 sequence was analyzed by dideoxynucleotide chain termination sequence method (Promega, Biotec). The sequence was also confirmed in part by the method of Maxam and Gilbert.

RESULTS

(a) Hybrid selection of the DNase encoding clone

The different cDNA clones derived from mRNAs of P3HR-1 cells expressing early EBV antigens were used to select mRNAs from induced P3HR-1 cells. The selected mRNAs were translated in a reticulocyte lysate system, and the peptides were tested for their ability to degrade ^{32}P-labelled DNA. One clone, c37, selected

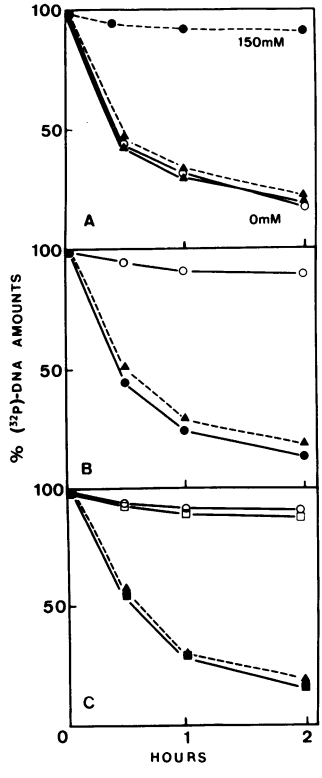

Figure 1 : Degradation of ^{32}P-labelled DNA by viral and cellular DNases
A - Endogenous reticulocyte DNase. Enzyme activity ▲——▲ is strongly inhibited by 150mM KCl ●---● but is unaffected by EA$^+$ O——O or EA$^-$ VCA$^+$ ▲---▲ sera.
B - DNAse translated from c37 hybrid selected mRNA from P3HR-1 cells. Enzyme activity ●—● mesured in the presence of 150mM KCl is inhibited by EA$^+$ serum O—O but not by EA$^-$ VCA$^+$ serum▲---▲.
C - Translation products from c37 nRNA. Product from the T7 promotor ■——■ but not that from the SP6 promotor □——□ has DNase activity which is inhibited by EA$^+$O—O but not by EA$^-$ VCA$^+$▲---▲serum.

a mRNA which translated to a typical EBV DNase which digested labelled DNA efficiently at 150 mM KCL and was inhibited by antiserum to EA but not by EA$^-$, VCA$^+$ serum (Fig. 1B). By contrast, reticulocyte DNase was almost totally inhibited by 150 mM KCl, but was unaffected by either EA$^-$ or EA$^+$ sera (Fig. 1A). Polyacrilamide gel electrophoresis of the cell-free translation products shows several bands, one of which, 52 Kd, is specifically precipitated by high titre anti-EA serum (Fig. 2, bands A,a). A VCA$^+$ EA$^-$ serum precipitated nothing from this mixture (data not shown)

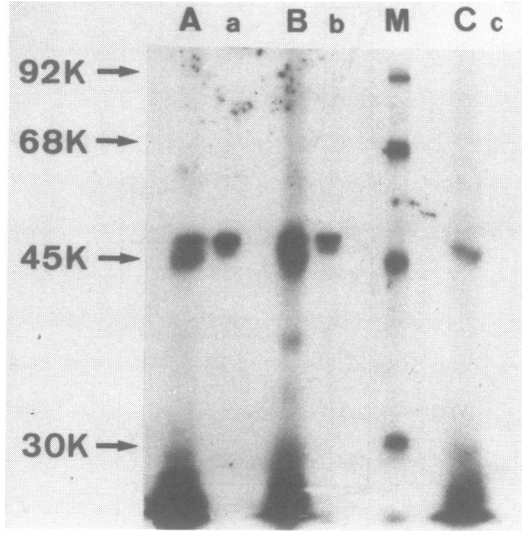

Figure 2. : Analysis of the cell-free translation products. Hybrid-selected mRNA or native RNA (nRNA) synthesized from the c37 cDNA sequence were used to direct the reticulocyte cell-free translation system and the products were analyzed on a $NaDodSO_4$ 10 % polyacrylamide gel before or after immunoprecipitation with EA positive serum. (A) Hybrid-selected mRNA : (a) Hybrid-selected mRNA with EA serum ; (B) nRNA on the left strand of pGEM-37 from T7 promotor : (b) the same with EA serum : (C) nRNA on the right strand of pGEM-37 from SP6 promotor : (c) the same with EA serum ; (M) Standard molecular sizes as indicated on the left side.

(b) Production and translation of RNA from c37

To investigate whether the c37 cDNA sequence contained a complete DNase gene, we cleaved it with Pst-1 and inserted it into the pGEM^{TM}-1 plasmid. Two kinds of capped native RNA (about 1.5 kb) were synthesized in vitro, one from the T7 promoter, which has the same direction as the left strand of EBV genome, and the other from the SP6 promoter which has the opposite direction (Figure 3D). These RNAs were translated in vitro and we tested the produced protein for DNase activity. The protein translated from the RNA origination from the T7 promotor digested labelled DNA in presence of 150 mM KCl and was specifically inhibited by anti-EA serum identically to the translation product from the hybrid selected P3HR-1 mRNA (Fig. 1C). The RNA produced from the SP6 promotor produced no DNase activity on translation (Fig. 1C-open squares). PAGE shows that the translated product from the T7 promotor RNA corresponds to a polypeptide of 52 Kda which is precipitated by anti-EA serum (Fig. 2, lanes B,b) whereas the SP6 promotor RNA gives no precipitable product (Fig. 2, lanes C,c). The band at ca 50 Kda which

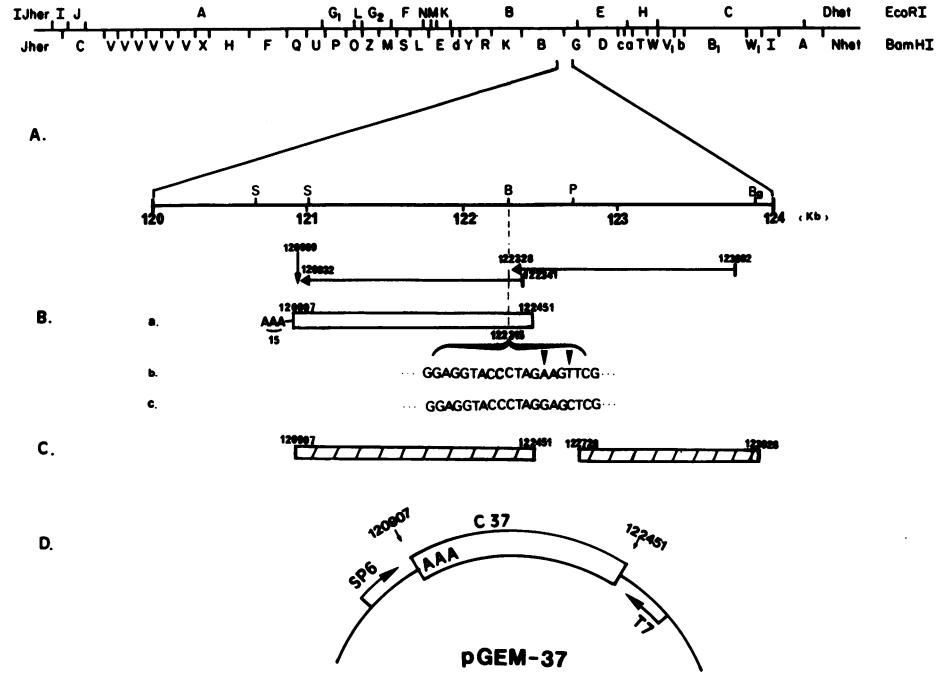

Figure 3. : Localization of c37 cDNA in EBV genome. (A) Schematic diagrams of the B95-8 EBV genome (1) and the open reading frame of BGLF4 [123692 to 122323 position] and BGLF5 [122341 to 120932 position] determined by C. Seguin et al. [1983]. Restriction endonuclease site abbreviations : S = Sac II ; B = Bam HI ; P = PstI ; Bg = Bgl II.(B) (a) Localisation of c37. Its two extremities are indicated with the number of base pairs corresponding to known B95-8 EBV genome [Baer et al., 1983]. (b) Sequence of the c37 near the Bam HI site at position 122315. Two sequence alterations are comparised with that of the B95-8 strain are indicated by black arrows. (c) Sequence of B95-8 strain near Bam HI site in 122315 position. (C) Localization of two probes used for northern blots : c37 sequence in the left and a genomic fragment of B95-8 between PstI and Bgl II site of Bam HI G. (D) Construction of pGEM-37 plasmid.

appears in lanes A,B and C in this figure corresponds to a product from an endogenous reticulocyte mRNA.

(c) Localization of an open reading frame encoding a DNase gene in the EBV genome

c37 cDNA has previously been localized to Bam HI B and G fragments of the B95-8 genome by restriction enzyme mapping. Its precise location was found by determining both end sequences, about 100 bp from the 3' terminus and 200 bp from the 5' terminus and comparing these with the sequences of B95-8 strain as established by Seguin et al. (23). The c37 sequence showed a run of 15 adenylate

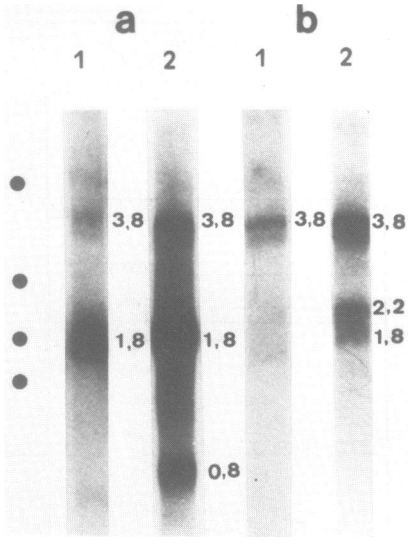

Figure 4. : Determination of the mRNAs transcribed from EBV genome between 120907 and 123928 positions. 10µg of polyA-RNAs from early stage (1) or late stage (2) expressing P3HR-1 cells were separated on 2.2mM formaldehyde gels, transfered onto Genescreen and hybridized to two probes (see fig. 3 C) : (a) with c37 (b) with a B95-8 genomic PstI-Bgl II fragment. The RNA size given in kilobases was determined by reference to eukaryotic (28 S, 18 S) and prokaryotic (23 S, 16 S) rRNA run on the same gel and indicated by (●)

residues at position 120907, a probable initiator ATG at 122341 and had its 5' terminus at 122451 (Figure 3Bc). For 100 bp from the 3' end its sequence was identical to that of B95-8 in that region, but at the 5' end some differences existed. Our 5' end sequence extends to the region of the B95-8 Bam HI site where it shows 2 nucleotide changes : Adenine, instead of Guanine at 122315 and Thymine instead of Cytosine at 122318, explaining the absence of Bam HI cleavage of P3HR-1 strain in this region (24). The size of our cDNA about 1.53 kb as determined on agarose gel electrophoresis was identical to that calculated from the corresponding B95-8 genomic sequence. This suggests that no major splicing occurs on transcription of this reading frame.

(d) RNA size

Northern blotting of mRNA from P3HR-1 cells limited to early viral expression with c37 clone as probe revealed 2 transcripts : one major at 1.8 kb and a minor 3.8 kb component. Virus-producing P3HR-1 cells contained RNAs of 0.8 ; 1.8 and 3.8 kb reactive with the c37 probe (Figure 4). The 0.8 kb RNA was detected only in productive cells and is a late messenger. The major early 1.8 kb RNA corresponds to

c37 cDNA open reading frame (ORF) and thus might be the DNase messenger. The 3.8 Kb RNA which is transcribed weakly during early stage but more strongly during the productive cycle is too large to be encoded by the c37 ORF. In order to determine the origin of the 3.8 Kb RNA, we examined an adjacent 1.2 Kb sequence situated upstream to the c37 ORF, corresponding to a PstI-Bgl II fragment of the B95-8 genome (Figure 3C). Using this sequence as a probe, a single major 3.8 Kb RNA was found in PAA-treated P3HR-1 cells, while two major RNAs : 3.8 Kb and 2.2 Kb were identified in productive cells. An additional very weak 3.5 Kb RNA was detected by both probes in both early and late viral stages in P3HR-1 cells. The PstI-Bgl II fragment also revealed a weak and indistinct band in the 1.8 kb region (Figure 4b2) during the late stage of viral expression.

DISCUSSION

Our studies show that the EBV genome encode an active DNase, supporting the findings reported by Littler et al. [10th International herpesvirus Worshop, Ann Arbor, Michigan, USA] and Mc Geoch et al. (14) on the presence of a weak amino acid sequence homology between a HSV-DNase gene and the Bam HI B and G regions of the EBV genome. The corresponding cDNA revealed an abundant RNA in P3HR-1 cells limited to EA expression, and the related enzyme activity was detected both in Raji cells and in P3HR-1 limited to early viral expression (8). This indicates that the gene is transcribed and translated after reactivation and during the early stage of the EBV cycle.

From the sequence analysis of both end of c37 cDNA, which allowad us to situate the AATAAA and ATG signals, we would predict that the DNase-coding cDNA sequence corresponds to the open reading frame BGLF5 of B95-8 genome described by Seguin et al. (23). The cDNA clone possessed an additional 110 bp upstream of the presumptive initiator ATG in which no TATA box-like sequence was identified (25), agreeing with previous reports (23). As expected from the origin of c37 clone (P3HR-1 strain), its sequence had no Bam HI cleavage site (24) and our sequence data on the corresponding region of our cDNA clone show two nucleotide changes in the B95-8 Bam HI site (23). These minor alterations in the c37 sequence did not affect the enzyme activity since they did not change the amino-acid sequence of BGLF5 ORF.

During the early stage of the EBV cycle, at least 2 polyadenylated cytoplasmic RNAs have been identified from c37 region. An abundant 1.8 kb transcript corresponds in size to the BGLF5 ORF (Figure 3A). Our data also imply that the BGLF4 ORF, situated upstream of BGLF5 may encode a late 2.2 Kb RNA since a probe from this region revealed no early RNA of the size of BGLF4 ORF, but a 2.2

Kb RNA was present in late-antigen-expressing P3HR-1 cells. The 0.8 kb RNA might be derived from BBLF1 which has been reported to encode a late RNA (13). Both BGLF4 and BGLF5 probes hybridized with a minor early 3.8 kb RNA whose transcriptional level increased in the late stage of viral expression. This RNA might correspond to the sum of the BGLF4 and BGLF5 ORFs. Recently, Hummel and Kieff (26) have mapped a large number of early and late B95-8 RNA transcripts to the Bam HI B and G regions. Among them, early 4.6 and 2.6 kb RNAs might correspond to our 3.8 kb and 1.8 kb mRNAs.

Although two early RNAs were recognized by a c37 probe, cell-free translation of its hybrid-selected RNAs produced a single 52 Kda protein which was recognized by EA positive serum. This corresponds well with the coding capacity of the 1.8 Kb mRNA from the BGLF5 ORF. An identical polypeptide was obtained by translation of mRNA obtained from c37 by the pGEM system. Hummel and Kieff (26) have localized an early 50 KDa protein to the Bam HI G fragment of B95-8 genome. Since the 52 KDa polypeptide obtained from pGEMI system has a functional DNase activity, the 1.53 kb c37 sequence must possess the sequence necessary for catalytic function. Although this present study did not permit to demonstrate the real molecular size of the viral DNase in vivo, we can nevertheless predict that a 52 KDa polypeptide would represent a form of DNase molecule as supported by the following data :

(a) a major 1.8 kb RNA which hybridized with our c37 cDNA clone, encoded an active 52 KDa DNase,

(b) 1.53 kb native RNA synthesized from our c37 cDNA sequence translated an active 52 KDa protein,

(c) EBV-specific DNase activity extracted from EBV early antigen positive lymphoid cells has a sedimentation coefficient of 4S on neutral sucrose gradients (8).

We cannot however exclude the possibility that the 52 KDa polypeptide is only a part of the DNase molecule and that other molecules are required for its function as the virus specific enzyme. Specific antibody against this protein is required for more detailed studies. As expected from the previous data (7,8), the DNases translated in vitro from either c37 hybrid-selected RNA or nRNA of pGEMI system were precipitated by EA positive serum from NPC patients, and their activities were completely neutralized in the presence of the serum, confirming that viral DNase is an element of the EA complex.

Our data indicate that the 1.53 kb c37 cDNA sequence encodes an active EBV-specific DNase with the expected biochemical and immunological properties. These findings may permit us to develop more specific tests for the prognosis of NPC, and for the further characterization of this enzyme.

ACKNOWLEDGEMENTS

This work was financially supported by the Centre National de la Recherche Scientifique (UM 30 & ATP 950079), the Association pour la Recherche sur le Cancer (contract nº 6125) and the Ministère de la Recherche & de la Technologie (contract nº 508420). We owe sincere thanks to Dr. E. Kieff (The University of Chicago) for criticism of the manuscript. We also thank Dr T. Greenland (Unité de Virologie, Lyon) for his assistance in the preparation of the manuscript and C. Weiler for secretarial work.

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REFERENCES

1. Kieff E., Dambaugh T., Heller M., King W., Cheung A., Van Santen V., Hummel M., Beisel C., Fennewald S., Hennessy K. and Heineman T. (1982) J. Infect. Dis., 146, 506-517.
2. de Thé G. (1982) In The Herpesviruses, ed. Roizman B. (Plenum Publishing Corp. New York) Vol. 1, pp 25-103.
3. Ooka T. (1985) Biomed. and Pharmacoth., 39, 59-66.
4. Zur Hausen H., O'Neill F.J., Freese U.K. and Hecher E. (1978) Nature, 272, 373-375.
5. Salmundsen A.K., Kallin B. and Klein G. (1980) Virology,107, 557-561.
6. Clough W. (1979) Biochemistry, 18, 4517-4521.
7. Cheng Y.C., Cheng J.Y., Hoffman P.J. and Glaser R. (1980) Virology, 100, 334-338.
8. Ooka T., de Turenne M., de Thé G and Daillie J. (1984) J. Virol., 49, 626-628.
9. Francke B. and Garrett B. (1982), Virology, 116, 116-127.
10. Resnick M.A., Sugino A., Nitiss J. and Chow T. (1984) Mol. Cell. Biol., 4, 2811-2817.
11. Strobel M. and Francke B. (1980) Virology, 103, 493-501.
12. Hoffmann M. and Francke B. (1980) Virology, 103, 493-501.
13. Baer R., Bankier A., Biggin M., Deininger P., Farrell P., Gibson T., Hatfull G., Hudson G., Satchwell S., Seguin C., Tuffnell P. and Barrell B. (1984) Nature, 310, 207-211.
14. Mc Geoch D.J., Dolan A. and Frame M.C. (1986) Nucl. Acids res., 14, 3435-3448.
15. Manet E., Chevallier A., Zhang C.X., Ooka T., Chavrier P. and Daillie J. (1985) J. Virol., 54, 608-614.
16. Ooka T. and Calender A. (1980) Virology, 104, 219-223.
17. Ooka T., Calender A., de Turenne M. and Daillie J. (1983) J. Virol., 46, 187-199. Resnick M.A., Sugino A., Nitiss J. and Chow T. (1984) Mol. Cell. Biol., 4, 2811-2817.
18. Henle G. and Henle W. (1966) J. Bacteriol., 91, 1248-1256.
19. Van Santen V., Cheung A., Hummel M. and Kieff E. (1983) J. Virol., 46, 424-433.
20. Maniatis T., Fritsch E. and Sambrook J. (1982) Molecular cloning. pp 162. Cold Spring Harbor Laboratory. Cold Spring Harbor, N. Y.
21. Laemmli (1970) Nature, 227, 680-684.
22. Maxam A. and Gilbert W. (1980) Methods in Enzymol., 65, 499-560.
23. Seguin C., Farrell P. and Barrell B.G. (1983) Mol. Biol. Med., 1, 369-392.
24. Heller M., Dambaugh T. and Kieff E. (1981) J. Virol., 38, 632-648.
25. Corden J., Wasylyk B., Buchwalder A., Sassone-Corri P. and Kedinger L. (1980) Science, 209, 1406-1414.
26. Hummel M. and Kieff E. (1982) Proc. Natl. Acad. Sci. U.S.A., 79, 5698-5702.